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B.V.V. Prasad, Prof. of Biochemistry, Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, Texas  
vprasad@bcm.tmc.edu

## X-ray Structure of the Rotavirus Protein Involved in Genome Replication and Packaging

H. Jayaram\*, Z. Taraporewala†, J. T. Patton† and B.V.Venkataram Prasad\*

\*Program in Structural and Computational Biology and Molecular Biophysics, Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, Texas; †Laboratory of Infectious Diseases, National Institutes of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland

*Using x-rays at beamline X8C of the National Synchrotron Light Source, scientists have determined, to 2.6 angstrom (tenth of a billionth of a meter) resolution, the first x-ray structure of a key protein, called non-structural protein 2 (NSP2) of a rotavirus, the major cause of life-threatening infantile gastroenteritis. The structure provides molecular details of the protein's doughnut-shaped functional part, and reveals how it may be involved in the replication and packaging of the viral RNA during infection.*

Rotavirus, the leading cause of infantile gastroenteritis, with nearly a million deaths worldwide every year, has a spherical shape with many spikes that bind firmly to a host cell. The virus is composed of six structural proteins arranged in three concentric layers, which enclose the viral genome consisting of 11 double-stranded RNA (dsRNA) segments. Each segment codes for at least one protein, except for one segment that codes for two proteins.

Every rotavirus particle is a fully contained unit that transcribes genome segments from the inside, and then releases the resulting messenger RNA (mRNA) molecules. When a rotavirus fully replicates its genome, several copies of the 11 segments are made (genome replication), and each progeny virus gets one copy of each segment (genome packaging).

One of the least understood, yet critically important, processes in the replication process of a dsRNA virus is

the genome replication and packaging. The process by which the correct set of dsRNA segments is assigned to the capsids of each progeny virus remains a mystery.

Several *in vivo* and *in vitro* studies on rotavirus have provided insight

into the role of proteins that are not incorporated into the virus but merely assist in genome replication and packaging. These proteins, referred to as non-structural proteins 2 and 5 (NSP2 and NSP5), are co-localized, along with the viral RNA polymerase, called VP1, in granular cytoplasmic inclusions, or viroplasms, in the infected host cell. (The RNA polymerase is the main protein involved in RNA replication.)

Recent biochemical studies have shown that NSP2's functional part, a doughnut-shaped octamer – containing eight repeating units, or monomers – binds to RNA, has a nucleoside triphosphatase (NTPase) activity and has nucleic acid destabilizing activity. Based on these studies, NSP2 is thought to work as a molecular motor involved in genome packaging, by using the energy derived from the hydrolysis of nucleoside triphosphates.

We have determined the crystal structure of the functional octamer of NSP2 to a



H. Jayaram (left) and B.V.V. Prasad



J. Patton (left) and Z. Taraporewala

resolution of 2.6 angstrom (tenth of a billionth of a meter) resolution (**Figure 1**). Our studies have provided a firm ground to develop a mechanistic understanding of how NSP2, in concert with NSP5 and VP1, may facilitate genome replication and packaging.

The structure of the NSP2 monomer (**Figure 2**) displays two distinct domains: an amino-terminal domain and a carboxyl-terminal domain. A characteristic feature of the monomer is a 25-angstrom deep cleft between both domains.

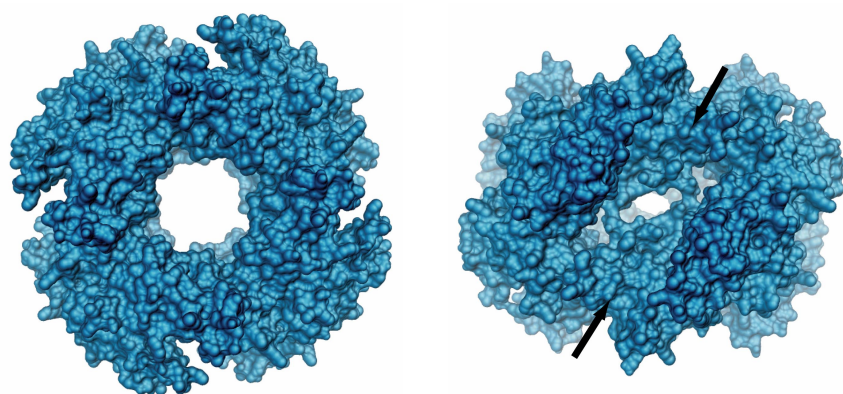
While the amino-terminal domain exhibits a fold not seen in any other

protein as yet, the carboxyl-terminal domain surprisingly, despite any noticeable sequence homology, exhibits a fold that is observed in the Histidine Triad proteins, a family of ubiquitous cellular proteins that hydrolyze nucleotides. This suggests that the cleft in NSP2 might correspond to the active site for NTP hydrolysis.

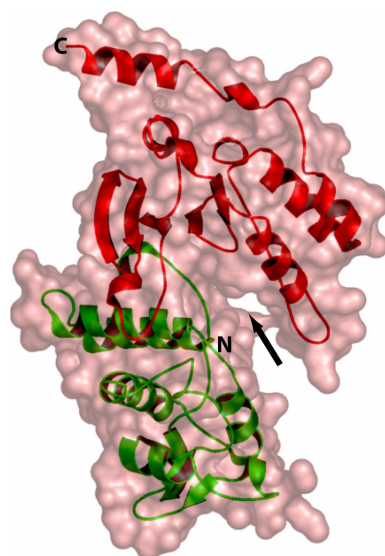
The RNA binding and nucleic acid helix destabilizing activities of NSP2 may require the formation of the octamer. In particular, prominent grooves, lined by positively-charged residues, at the sides of the octamer are ideal for binding to single-stranded RNA. The pro-

posed NTP binding sites are located on either side of the groove.

Although NTP hydrolysis is not directly linked to either the RNA binding or the helix destabilizing activity of NSP2, nucleotide binding into the cleft may alter the conformation of the monomer and affect octamer-RNA interactions. Such changes may facilitate transfer of the bound RNA through the polymerase during replication, resulting in concurrent synthesis of duplex RNA and its packaging into assembling progenitor viruses.



**Figure 1.** Surface representations of the octameric part of the non-structural protein 2 (NSP2). (Left): View along the 4-fold axis of the octamer's 4-2-2 symmetry. (Right): View along one of the 2-fold axes. The groove in the octamer is shown by a pair of arrows.



**Figure 2.** X-ray structure of the monomeric subunit of the non-structural protein 2 (NSP2). The amino- and carboxyl-terminal domains are shown in green and red, respectively. The cleft between the domains is indicated by an arrow.